Open Access
J. Glob. Innov. Agric. Sci., 2023, 11(2):199-203
ISSN (Online): 2788-4546; ISSN (Print):2788-4538
DOI: https://doi.org/10.22194/JGIAS/11.1059
http://www.jgiass.com

Impact of insect growth regulators on reproduction and life stages of *Tribolium Castaneum* (Coleoptera: Tenebrionidae)

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Tribolium castaneum is devastating cosmopolitan insect pest of stored grain. The present study investigated the impact of three insect growth regulators on different life stages of *T. castaneum*. Percent mortality (%) induced was observed at 0.75. 1.25, 2.5, 5, 10, and 20 ppm doses of each IGR after 7, 14 and 21 day exposure. In addition to larval and parental mortality, progeny suppression data was also recorded. The larvae of the 2nd instar were more susceptible to lufenuron, with mortality reaching 100% at 10ppm even after a 14-day interval. In the case of pyriproxifen, 2nd instar did not responded to all dose rates until 14 days however 21 days, statistically corresponding 2.5 to 10 ppm dose rates was detected. At 20 ppm, methoxy fenazide resulted in 92 percent mortality of 2nd larval instar mortality, and the larvae responded to all dose rates at all exposure intervals that varied significantly among themselves. The mean mortality of the 4th instar larvae at 20 ppm for lufenuron after a 21-day interval was 90%, which was not significantly different from the mortalities (62 and 80%) at the same dose rate after 7 and 14 days, respectively. All the IGRs were very effective in progeny suppression (>80%) and complete progeny suppression (100%) was observed in adults treated with methoxyfenazide and lufenuron at 2.5 and 10 ppm doses, respectively. It is concluded that all the screened IGRs were effective in progeny suppression and in some cases, complete progeny suppression was achieved
Key words: Exposure interval, life stages, IGRs, *Tribolium castaneum,* wheat flour

INTRODUCTION

Storage losses caused by insect pests have been assessed at about 10 to 20% in Pakistan (Khan *et al.,* 2010). Lack of sanitary conditions during transport and storage; leaky and destructed godowns (Alamet *et al*., 1984; Ahmed *et al*., 19991); storage of low quality infested grains; inadequate safety measures; poor and improper application of insecticides and fumigants; and a shortage of skilled labor are the major causes of such high storage losses (Ahmed *et al*., 2008). Damage of grain due to infestations of insects, mites, and fungi is the main post-harvest source of disturbing the dietary value and market worth of stored grains (Ahmed *et al.,* 2008). The key pests that damage stored grain are *Trogoderma granarium* (E), *Rhyzopertha dominica* (F), *Tribolium castaneum* (H) and *Sitophilus oryzae* (L.). These insects are major causes of deterioration of food products with fragments of insects' bodies and related contaminants which are allergic and cancer-causing (Hubert *et al.,* 2018). *Tribolium castaneum* is the most damaging cosmopolitan insect pest in stored grain *(*Fedina and Lewis, 2007) and it can be found in a wide range of food processing mills. Grubs and adults cause damage to all types of damaged stored grains, including wheat flour and dry fruits. Both adults and larvae usually do not damage the whole grains but only feed on grains already damaged by other primary pests (Sharaby, 1988).

Several nonchemical control measure viz. Phosphine fumigation (Carpaneto *et al*., 2016), diatomaceous earth (Korunic, 1998; Kavallieratos *et al*., 2015, 2018), and the use of pyrethroid insecticides (Ghimire *et al*., 2016; Arthur *et al*., 2018) are traditionally used to control the stored grain insects. Insecticide resistance (Boyer *et al*., 2012; Zettler and Cuperus, 1990); the presence of insecticide residues in grains

Malik, H., Q. Ali, G. Murtaza, A. Aslam, N. Din, M.J. Saleem, A. Abbas, S. Jamil, M.S. Hanif, T. Nazir, K. Hanif, M.F. Akhtar and N.A. Anjum. 2023. Impact of insect growth regulators on reproduction and life stages of *Tribolium Castaneum* (Coleoptera: Tenebrionidae). Journal of Global Innovations in Agricultural Sciences 11:199-203.
[Received 7 Mar 2023; Accepted 14 Apr 2023; Published 27 Jun 2023]

(Phillips and Throne, 2010); broad spectrum modes of action that are harmful to both targeted and non-target organisms (Fields, 1992; Hagstrum and Subramanyam, 2006); and health risks (Arthur, 1996) are all constraints for synthetic insecticides' use on stored grains. There is documented evidence that at present, 15 species of stored grain insects have developed resistance against phosphine (Chaudhry 2000, Nayak et al., 2003). The recent ban on methyl bromide (Mebr), a liquid fumigant that was very effective against storage pests, has prompted research into various MeBr alternatives (Fields and White 2002).In addition to phosphine resistance, consumer demand for residue free food is a growing concern and a driving force behind the low-risk control of stored grain insects.

Safe alternate methods are necessary to overcome these problems for the management of stored grains insect pests. IGRs have been successfully used as alternatives to conventional products for the management of stored grain pests (Oberlander *et al*., 1997; Mondal and Parween, 2000). IGRs are harmless to humans and the atmosphere and are harmonious with IPM approaches (Staal, 1975). These products are highly compatible for food security compliance (Phillips and Throne, 2010). The IGRs control the pest species through inhibiting growth, development and emergence of insects and are friendly for the non-target organisms than most of the conventional grain protectants (Oberlander *et al.,* 1997; Oberlander and Silhacek, 2000).

There are three types of IGRs on the basis of their mode of action. The first is chitin synthesis inhibitors that inhibit cuticle formation; the second is juvenile hormone agonists that interfere with growth and the development of immature young insects or the stimulus of metamorphosis (Oberlander et *al.,* 1997; Oberlander and Silhacek, 2000) and the third one is ecdysteroid agonists that cause the synthesis of the insect's cuticle in premature stages of growth and feeding inhibition (Schneiderman, 1972; Fox, 1990; Wing and Aller, 1990). These compounds enter the insect's cuticle (Schneiderman, 1972) and have chemo-sterilant activity in females (Heller *et al*., 1992) through the stomach and contact (Fox, 1990). A number of studies have been conducted toevaluate the potential of IGRs against insect pests of stored-grain (Arthur *et al.,* 2018; Yasir *et al.,* 2019; Trostanetsky *et al.,* 2015; Ali *et al*., 2016, 2017, 2018; Malik *et al.,* 2017; Arthur and Hartzer, 2018).The use of IGRs considerably affects the reproductive development of insects. Mortality of insect pests in stored grains caused by inhibiting the biosynthesis of chitin (Tunaz and Uygun 2004). Use of IGRs atyoung immature stages prevents the normal emergence of adults and causes sterile adult emergence (Ware 2000). IGRs cause a substantial decrease in thlarval, pupal and adult populations of *T. castaneum* thus being responsible for progeny suppression (Awais, *et al.*2019)

The purpose of this study was to investigate the toxicity and potential efficiency of insect growth regulators on different life stages and progeny of the *Tribolium castaneum* in wheat flour.

METERIALS AND METHODS

***Insect Culture*:** The life stages that were studied in the present study were obtained from the laboratory reared population of *T. castaneum* at the Entomological Research Institute, Ayub Agriculture Research Institute, Faisalabad, Pakistan. Insect culture was reared in wheat flour containing 10% Brewer's yeast under laboratory conditions at temperature 30 ± 2 ºC and 60 ± 5% RH.

***IGRs Formulations*:** The tested IGR formulations used in the present study were Poker® comprising 10.8% EC Pyriproxifen (provided by Auriga), Match® comprising 5%EC Lufenuron (AI) (provided by Syngenta) and Runner® comprising 240SC Methoxyfenazide (provided by Arysta Pakistan Limited).

***Commodity and stock solution*:** Untreated and previously uninfested wheat flour were mixed with 10% Brewer's yeast was used in the tests. Each IGR formulation was made into a 50 ppm stock solution using acetone as the solvent. The stock solution was further diluted to get the desired concentrations of 20, 10, 5, 2.5, 1.25 and 0.75ppm. All the treatments, including control (acetone only) were replicated five times.

Bioassays

Against larvae: Fifty pairs of uniform age of *T. castaneum* adults were released in 200gm of wheat flour with 10% Brewer's yeast in a 9:1 ratio. Larvae of 2nd and 4th instar were obtained after 6 and 21 days, respectively, by using 60 mesh sieve. About 20gm of wheat flour with 10% Brewer's yeast was added to the petri plate of each replicate and sprayed with 2ml of different (20, 10, 5, 2.5, 1.25 and 0.75ppm) IGRs concentrations with the help of a fingertip sprayer. Control was treated with acetone only. After about 3 hours, when all the acetone was evaporated from the treated flour, twenty 2nd and 4th instar larvae were added into a separate petri plate of each replicate. The petri plates containing treated wheat flour and larvae were put in an incubator at 30 ± 2 ºC, 60 ± 5% RH. Percent larval mortality was recorded at 7, 14 and 21 day intervals.

Against adults: Bioassay studies for adults were similar to those for larvae. Thirty-five plastic jars of 250 gm. capacity were prepared by adding 20 gm. of wheat flour (10% Brewer's yeast) and treated with IGRs at the dose rates of 20, 10, 5, 2.5, 1.25 and 0.75ppm. Ten pairs of *T. castaneum* adults were added to plastic jars containing wheat flour. The jars' mouths were wrapped with muslin cloth. All the jars were then shifted into an incubator at temperature 30 ± 2 ºC and 60 ± 5% RH. Adults were observed for mortality after 7, 14 and 21 days. After an exposure period of 21 days, the dead insects were removed from the jars and the surviving adults were left in wheat flour for 2 weeks for progeny emergence. After about 2 weeks, adults were removed from the jars and

progeny were counted in treated and untreated jars to get the progeny reduction (%).

Statistical analysis: Larval and adult mortality data were corrected where necessary by using (Abbott 1925) formula. To fit the assumption of ANOVA and normally distributed in nature, mortality (%) data of 2nd and 4th instar larvae were transformed to arcsine square root and adults mortality (%) data were transformed to square root $\sqrt{(0.5+x)}$. To compare the means between dependent variables Wilk's Lambda test of multivariate repeated measures ANOVA was estimated using IBM SPSS statistics 20.0. Graph Pad prism 6.02 was used to perform Linear Regression Analysis to study Dose-Response Modelling for Different Life Stages at 7, 14 and 21 day intervals. Mortality means were isolated by Tukey's honest significant (HSD) test at a significance level of 0.05 (Sokal, 1995) The Progeny reduction percentage calculated by (Aldryhim 1990) is given as:

$$\text{Progeny Reduction (\%)} = \frac{\text{No.progeny in control} - \text{No.progeny in treatment}}{\text{No.progeny in control}} \times 100$$

RESULTS

The main effects (for exposure, df = 2, F = 454.5, P < 0.05; IGR, df = 2, F = 732.330, , P < 0.05; dose, df = 5, F = 250.52, P < 0.05; life stage, df = 2, F = 986.686, P < 0.05) and related interactions (exposure * life stage * IGR * dose, df = 40, F = 2.02, P < 0.05; exposure * IGR * dose, df = 20, F = 2.363, P < 0.05; exposure * life stage * IGR, df = 8, F = 8.669, P < 0.05; exposure * life stage * dose, df = 20, F = 3.078, P < 0.05; exposure * IGR, df = 4, F = 61.792, P < 0.05; exposure * dose, df = 10, F = 16.891, P < 0.05; exposure * lifestage, df = 4, F = 11.512, P < 0.05; lifestage * IGR * dose, df = 20, F = 4.601, P < 0.05; IGR * dose, df = 10, F = 41.237, P < 0.05; life stage * IGR, df = 4, F = 72.893, P < 0.05; life stage * dose, df = 10, F = 7.264, P < 0.05) were significant for mortality levels of different life stages of *T. castaneum* treated with different doses of tested IGRs. MANOVA estimates of repeated measures ANOVA is presented in Table 1.

All the tested formulations of IGRs showed significant insecticidal effects against the exposed life stages, recorded at different doses and exposure intervals (Fig. 1). The larvae of the second instar were more susceptible to lufenuron, with mortality reaching 100% at 10ppm even after a 14-day interval. In the case of pyriproxifen, second instar larvae did not respond to all dose rates until 14 days, and after 21 days, statistically non-significant differences in mean mortality (4-10%) at 2.5 to 10 ppm dose rates were detected. At 20 ppm, methoxyfenazide resulted in 92 percent of second larval instar mortality, and the larvae responded to all dose rates at all exposure intervals that varied significantly among themselves.

Table 1. MANOVA estimates of repeated measures ANOVA for main and related interaction of *T. castaneum* different life stages against IGRs.

Within exposure intervals	df	F	P
Exposure	2	454.50	<0.05
Exposure * lifestage * IGR * dose	40	2.02	<0.05
Exposure * IGR * dose	20	2.36	<0.05
Exposure * lifestage * IGR	8	8.67	<0.05
Exposure * lifestage * dose	20	3.08	<0.05
Exposure * IGR	4	61.79	<0.05
Exposure * dose	10	16.89	<0.05
Exposure * lifestage	4	11.51	<0.05
Between exposure intervals			
Intercept	1	9301.35	<0.05
Lifestage * IGR * dose	20	4.60	<0.05
IGR * dose	10	41.24	<0.05
Lifestage * IGR	4	72.89	<0.05
Lifestage * dose	10	7.26	<0.05
IGR	2	732.33	<0.05
Dose	5	250.40	<0.05
Lifestage	2	986.69	<0.05
Error	216		

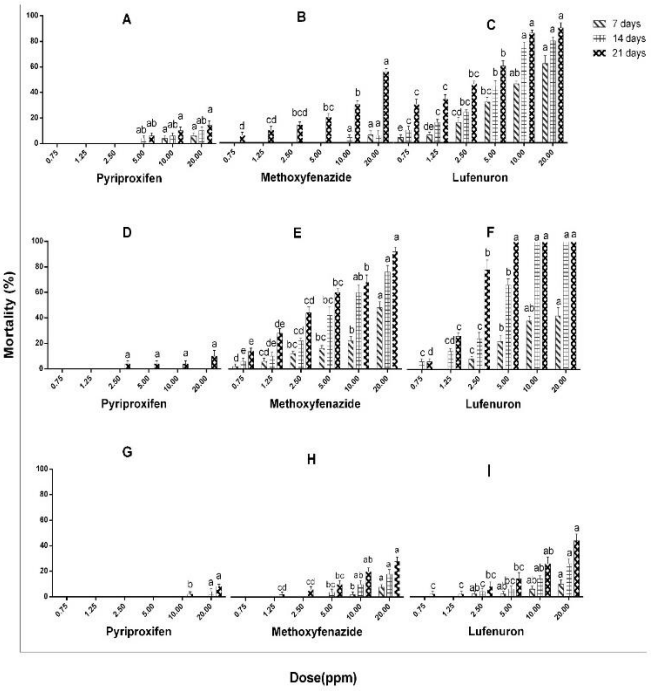

Figure 1. Mean mortality percentage ±standard errors of *T. castananeum* life stages exposed to wheat flour treated with 20, 10, 5, 2.5 1.25 and 0.75 ppm of IGRs at 7, 14 and 21 day interval. Means and SE are on back transformed data. Means sharing the same letter are not statistically significant at P = 0.05. where; A, B, C = 4th instar larvae, E, F, G = 2nd instar larvae and G, H, I = adults.

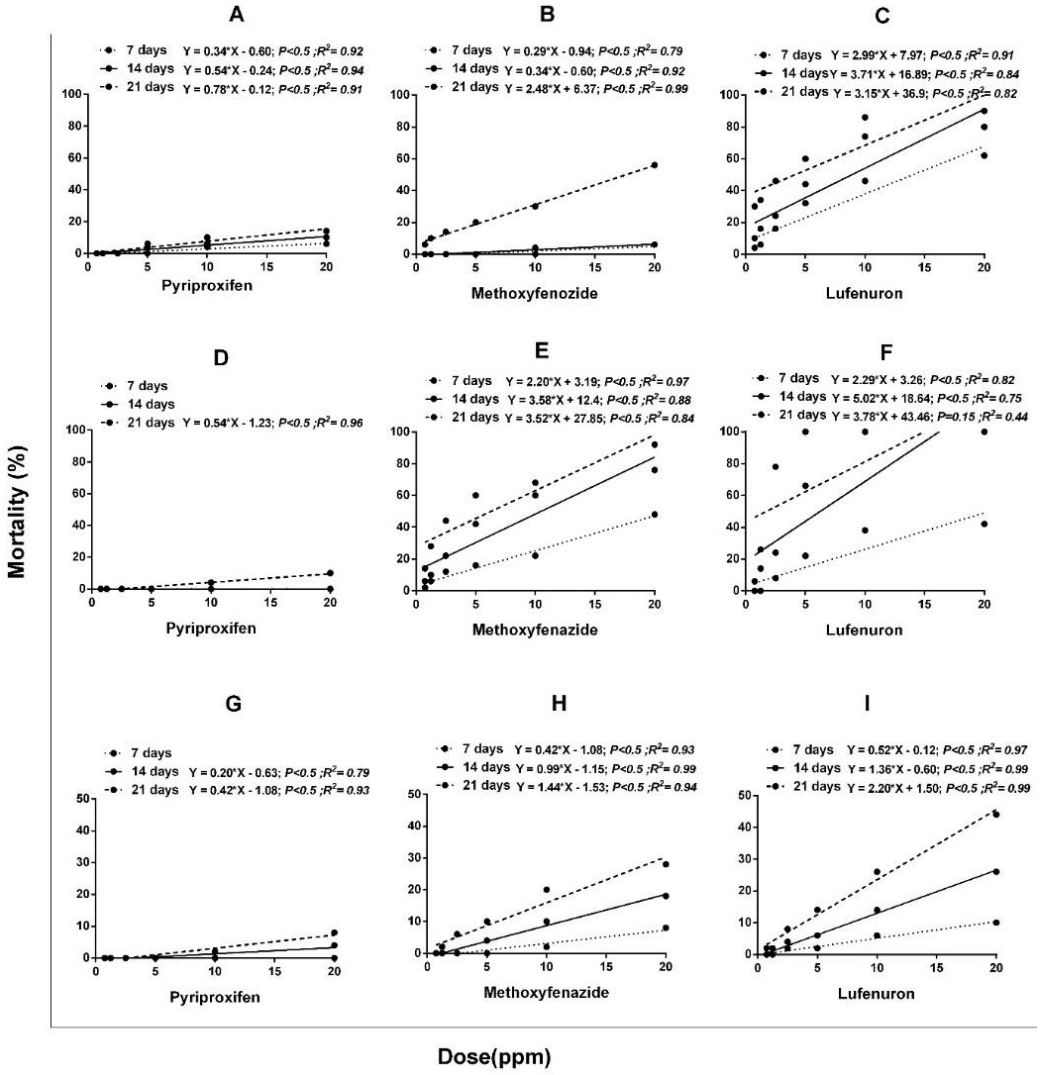

Figure 2. **Dose response mortality lines of *T. castaneum* 2nd, 4th instar larvae and adults exposed to different (20, 10, 5, 2.5, 1.25 and 0.75µl/l) dose rates for 7, 14 and 21 days interval. Where, R^2 = coefficient of variation, X = dose (ppm), Y = response mortality (%).**

The mean mortality of the fourth instar larvae at 20 ppm for lufenuron after a 21-day interval was 90%, which was not significantly different from the mortalities (62 and 80%) at the same dose rate after 7 and 14 days, respectively. In the adult bioassay, the dose rates and exposure intervals of all the IGRs were not enough to reach the end point mortality. Pyriproxifen and methoxyfenazide showed very little response (8 and 28%) against adults, respectively. In case of lufenuron treated diet, the highest mortality was 44% at 20ppm after 21 days exposure interval.

A Linear regression model designed to indicate the amount of variation (R^2) in the dependent variable (Y = mortality %) due to the independent variable (X = dose in ppm) is presented in Fig. 2. Dose response mortality lines for larval and adult stages depicted a significant association ($p < 0.05$) with the exception of 2nd instar larvae mortality response at 21 day exposure period ($P = 0.15$). The regression model was unable to determine dose response relationship in case of pyriproxifen applied to 2nd instar larvae (7 and 14 day intervals) and adults (7 day interval) as, no mortality was recorded at these intervals.

Of the tested IGRs formulations lufenuron and methoxy fenazide successfully inhibited F_1progeny production (%) of treated adults from 79 to 100% (Fig. 3). Among the IGRs tested here, lufenuron was the most efficacious since it inhibited the F_1progeny (81-100%). The less effective IGR tested in the study was pyriproxifen, causing 39.36 to 80.67% progeny reduced.

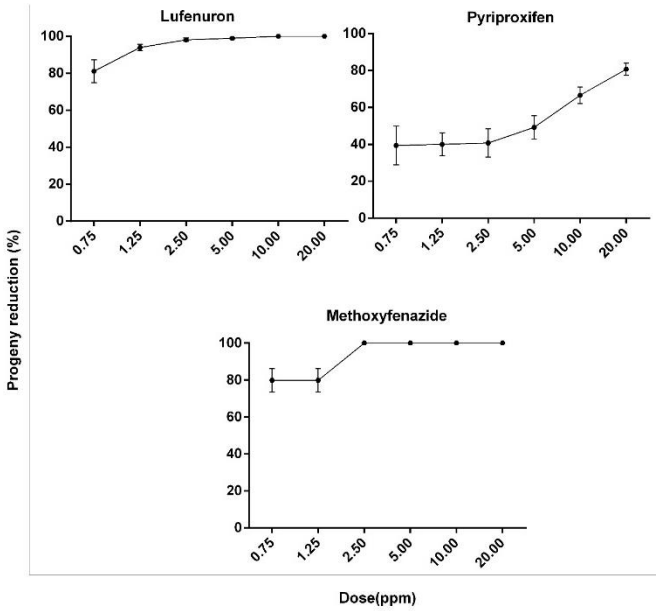

Figure 3. F_1 progeny reduction % (Means ± SE) of *T. castaneum* adults treated with 20, 10, 5, 2.5, 1.25 and 0.75 ppm doses of IGRs after 5 weeks interval.

DISCUSSION

Life stages of *T. castaneum* that were used as study models to screen out the tested IGRs indicated greater variability of response to IGRs diluted concentrations and the time span for which the developmental stages were exposed. The findings of the present study revealed that the tested life stages started showing response at ≥ 5ppm dose and gradually peaked towards high dosing. In our results, the highest mortality was by lufenuron application, which is a chitin synthesis inhibitor analogue and its effectiveness is due to contact and stomach mode of action (Hammann and Sirrenberg, 1980; Fox, 1990; Wang *et al.,* 1994). Despite the fact that lufenuron was the most effective of the IGRs tested in the current study, its efficacy against adults was only 44 4% at 20 ppm dose and 21 day interval.It is documented in the previous studies that IGRs effects are more pronounced on the immature life stages of insect pests and have very little effect on adults (Mohandass, *et al*., 2006, Oberlander, *et al*., 2000, Ali, *et al*., 2016).

Pyriproxifen was more active against 4th instar larvae as compared with 2nd instar larvae. This is probably due to the fact that juvenile hormone analogue targets during this critical stage of late 4th instar larvae or early 5th instar larvae when ecdysteroid concentration is at its peak (Thomas and Bhatnagar-Thomas, 1968; Tunaz and uygun, 2004). After 21 days, the mean mortality rate caused by pyriproxifen was only 82% in adults. Contradictory results have been reported by Kavallieratos. Nickolas G., *et al.,* 2012, where 13.8 ± 3.4% adult mortality was achieved by pyriproxifenin *P. truncates* at 10ppm and 14 days post exposure period. The difference might be due to the different insect pests studied. The results are also not in agreement with Ali, *et al*, 2016. who, reported no effect of pyriproxifen on adult mortality.

Methoxy fenazide also resulted in significant larval mortality and the response was more quick and high against the early instars as compared with late instar larvae (Oberlander *et al*. 1997). Again, the adults were less responsive (28 ± 4%) to methoxy fenazide (Kavallieratos, *et al*., 2012).

The use of IGRs against *T. casataneum* adults provided substantive evidence for progeny suppression. All the applied IGRs were effective, with more than 80% progeny reduction. For instance, lufenuron-treated commodities caused 81-100% progeny suppression of treated adults A completely complete reduction of F_1 progeny produced was achieved at a 10ppm dose. Kavallieratos. *et al*., 2012 found that wheat commodities treated with 10ppm dose of lufenuron inhibited 100% F_1 progeny production of *R. dominica* adults. Interesting results were obtained by methoxy fenazide which inhibited100% progeny production at 2.50ppm. Slightly different findings were revealed by Kavallieratos, *et al.,* 2012 who discovered 94.4, 96.4 and 96.4% progeny suppression of *P. truncatus* adults on maize treated with doses of 1, 5 and 10ppm. Pyriproxifen was less effective in achieving the 100% response in progeny reduction, with a maximum of 80% progeny reduction at high dose of 20ppm.

***Conclusion*:** It is concluded that the age and life cycle stage of an insect play an important role in the effectiveness of treated IGRs. The mode of action and dosing is another decisive factor playing a significant role in controlling the effects of IGRs. Immature stages were more responsive to IGRs than mature stages and adults. All the screened IGRs were effective in progeny suppression and in some cases, complete progeny suppression was achieved even with 2.5ppm suggesting that IGRs can be used as potential grain protectants against stored grain insect pests. As a result, more research is needed to investigate the effects of IGRs combined with low-risk insecticides in providing long-term stored grain pest protection

***Authors' contributions*:** H.M. plan the study and wrote the manuscript, Q.A. Supervised in planning and execution of this study, G.M. Supervised the execution of the experiment, A.A. Performed the statistical analysis, arrange the manuscript according to the journal requirements and corresponding author of the manuscript, M.J.S. Critical review and proof read the manuscript., N.D. and A.A. Collected the data and wrote the initial draft of the paper, S.J. and M.S.H. Designed structure of the manuscript, T.N. and K.H. Give technical inputs for improvement of the experiment, M.F.A. and N.A.A. provide the helpful material and assist in data recording and writing of manuscript.

***Funding*:** There is no funding agency for this study

***Ethical statement*:** The authors declare that there is no conflict of interest

***Availability of data and material*:** Data will be available on realistic request

Acknowledgement: Not Applicable

Code Availability: Not Applicable

***Consent to participate*:** All authors are participating in this research study

***Consent for publication*:** All authors are giving the consent to publish this research article in JGIAS

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